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INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference 20763WO	**FOR FURTHER ACTION**	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
International application No. PCT/EP 03/05726	International filing date *(day/month/year)* 28.05.2003	Priority date *(day/month/year)* 30.05.2002
International Patent Classification (IPC) or both national classification and IPC C12N9/40		
Applicant DSM IP ASSETS B.V. et al.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 6 sheets, including this cover sheet.

   ☐ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the opinion
   - II ☐ Priority
   - III ☒ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☐ Lack of unity of invention
   - V ☒ Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
   - VI ☐ Certain documents cited
   - VII ☐ Certain defects in the international application
   - VIII ☐ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
05.11.2003	25.06.2004
Name and mailing address of the international preliminary examining authority: European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized Officer Guarinos Viñals, E Telephone No. +49 89 2399-7228

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/EP 03/05726

I. Basis of the report

1. With regard to the **elements** of the international application *(Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17))*:

Description, Pages

1-55 as originally filed

Claims, Numbers

1-21 as originally filed

Sequence listing part of the description, pages:

1-178, as originally filed

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

   These elements were available or furnished to this Authority in the following language: , which is:

   ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).

   ☐ the language of publication of the international application (under Rule 48.3(b)).

   ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

   ☒ contained in the international application in written form.

   ☒ filed together with the international application in computer readable form.

   ☐ furnished subsequently to this Authority in written form.

   ☐ furnished subsequently to this Authority in computer readable form.

   ☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

   ☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

   ☐ the description, pages:

   ☐ the claims, Nos.:

   ☐ the drawings, sheets:

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)).

   (Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)

6. Additional observations, if necessary:

III. Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

1. The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non-obvious), or to be industrially applicable have not been examined in respect of:

☐ the entire international application,

☒ claims Nos. 1-21 partially

because:

☐ the said international application, or the said claims Nos. relate to the following subject matter which does not require an international preliminary examination (specify):

☐ the description, claims or drawings *(indicate particular elements below)* or said claims Nos. are so unclear that no meaningful opinion could be formed *(specify)*:

☐ the claims, or said claims Nos. are so inadequately supported by the description that no meaningful opinion could be formed.

☒ no international search report has been established for the said claims Nos. 1-21 when concerning PEC2-PEC32

2. A meaningful international preliminary examination cannot be carried out due to the failure of the nucleotide and/or amino acid sequence listing to comply with the standard provided for in Annex C of the Administrative Instructions:

☐ the written form has not been furnished or does not comply with the Standard.

☐ the computer readable form has not been furnished or does not comply with the Standard.

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	8, 12, 14, 21
	No:	Claims	1-7, 9-11, 13, 15-20
Inventive step (IS)	Yes:	Claims	
	No:	Claims	8, 12, 14, 21
Industrial applicability (IA)	Yes:	Claims	1-21
	No:	Claims	

2. Citations and explanations

see separate sheet

Reference is made to the following documents:

D1: US-A-5 447 862 (MEYHACK BERND ET AL) 5 September 1995 (1995-09- 05).

D2: WO 94 14966 A (ROLIN CLAUS ;MUELLER YVONNE (NL); VISSER JACOB (NL); GIST BROCADES) 7 July 1994 (1994-07-07).

D3: MILL P J: 'The pectic enzymes of *Aspergillus niger*. A mercury-activated exopolygalacturonase.' THE BIOCHEMICAL JOURNAL. ENGLAND, vol. 99, no. 3, June 1966 (1966-06), pages 557-561.

D4: MILL P J: 'The pectic enzymes of *Aspergillus niger*. A second exopolygalacturonase.' THE BIOCHEMICAL JOURNAL. ENGLAND, vol. 99, no. 3, June 1966 (1966-06) pages 562-565.

Novelty (Art 33(2) PCT)

Document D1 discloses the polynucleotide sequence encoding the pectin lyase PLC from *Aspergillus niger* (SEQ ID NO:7) which shares 85.19% identity over 1303 bp with SEQ ID NO:33 from the present application (see examples 12.3, 12.4, 12.5 and 13.2).
Also disclosed in D1 are vectors and recombinant host cells, including the transformed *A. niger* strain A15, comprising said polynucleotide (see examples 14.1, 14.2 and 14.3).
In the light of the identity shared by the polynucleotide from D1 and the polynucleotide identified as SEQ ID NO:33 from the present application, the subject matter of claims 1-4, 9-11, 18 is not novel under Art 33(2) PCT.

Document D2 discloses the polynucleotide sequence encoding the exopolygalacturonase (PGX) from *Aspergillus tubigensis*. The PGX enzyme has been purified from *A. tubigensis* (see example 1.1). Also disclosed are strains of *A. niger*, *A. nidulans* and *A. tubigensis* transformed with the gene encoding the PGX enzyme (see example 4.1). The recombinant protein was detected by using polyclonal antibodies raised against the purified PGX protein (see example 4.2) and was purified afterwards from the *A. tubigensis* transformant (see example 4.3).
The present application discloses an exopolygalacturonase from *A. niger* identified by SEQ ID NO:65 and encoded by SEQ ID NO:33. Therefore, document D2 discloses a

functional equivalent of the polypeptide identified by SEQ ID NO:65 and consequently the subject matter of claims 5-7, 13, 15-17, 19, 20 is not novel under Art 33(2) PCT.

Inventive step (Art 33(3) PCT)

Document D2 discloses the *pgaX* gene encoding an exopolygalacturonase from *Aspergillus tubigensis*.

The difference between D2 and the present application is that, whereas D2 discloses the polynucleotide encoding an exopolygalacturonase from *A. tubigensis*, the present application discloses a polynucleotide encoding an exopolygalacturonase from *A. niger*.

Therefore, in the light of D2, the problem solved by the present application is the provision of a further polynucleotide encoding an exopolygalacturonase from *Aspergillus sp.*, the solution being the polynucleotide encoding an exopolygalacturonase from *A. niger* and identified by SEQ ID NO:33

The question to be answered is whether this solution is obvious in the light of the prior art.

On the one hand, documents D3 and D4 disclose the existence of two different exopolyglacturonases in *A. niger* and the methods to purify them.

On the other hand document D2 discloses the hybridization of the polynucleotide encoding the *pgaX* gene from *A. tubigensis* to the genomic DNA from *A. niger*, *A. tubigensis* and *A. nidulans*. According to the authors of D2, hybridizing fragments were found in all three strains using a fragment of the *A. tubigensis pgaX* gene as a probe. Therefore document D2 provides a strong evidence of the existence of a similar gene encoding an exopolygalacturonase in *A. niger*.

The solution provided by the present application is obvious in the light of the teaching of documents D2-D4 for the following reasons;

Starting from D3/D4, it would be obvious for a person skilled in the art to purify an exopolygalacturonase from *A. niger* as disclosed in any of these documents and to proceed afterwards as the authors of D2 did in order to identify the polynucleotide

sequence encoding it (i.e. determination of the amino acid sequence of several derived peptides, design of oligonucleotides corresponding to said peptides and screening of a genomic library by using them).

Optionally, starting from D2, a person skilled in the art would use the polynucleotide sequence encoding the exopolygalacturonase disclosed in D2 and, motivated by two strong evidences: the existence of an homologous gene in *A. niger* (see example 5 in D2 where hybridizing fragments were found in *A. niger* using a fragment of the *A. tubigensis pgaX* gene as a probe) and the existence of exopolygalacturonase activity in *A. niger*, isolate the polynucleotide sequence encoding the exopolygalacturonase from *A. niger*.

Therefore, the solution provided by the present application is obvious in the light of the prior art and consequently the subject matter of claim 8 is not inventive under Art 33(3) PCT.

The subject matter of claims 12, 14 and 21 embraces methods and products which are well-known in the art and which do not involve any inventiveness.

Consequently the subject matter of claims 12, 14 and 21 is also not inventive under Art 33(3) PCT.